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The cave beetle genus *Anthroherpon* is polyphyletic; molecular phylogenetics and description of *Graciliella* n. gen. (Leiodidae, Leptodirini)

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Abstract

The subtribe Anthroherponina form an iconic group of obligate cave beetles, typical representatives of the Dinaric subterranean fauna, which is considered to be the richest in the world. Phylogenetic studies within this subtribe are scarce and based only on morphological characters, which, due to troglomorphic convergence, are frequently unreliable. Moreover, morphological stasis and morphological polymorphism make classification of taxa difficult. To test if characters that have traditionally been accepted as informative for Anthroherponina classification are indeed reliable, we evaluated the monophyly of the most species-rich genus of this subtribe - *Anthroherpon* Reitter, 1889. Our study, based on a molecular phylogenetic analysis of fragments of the 18S, 28S, and COI (both 5' and 3' end) loci revealed that the genus *Anthroherpon* as conventionally defined is polyphyletic. To resolve this polyphyly, we defined one new additional genus, *Graciliella* n. gen., for which we then examined the intra-generic diversity using molecular and morphometric approaches. Molecular phylogenetic analysis of two COI mitochondrial gene fragments revealed the presence of four species inside *Graciliella* n. gen., including two new species, which we here describe as *G. kosovaci* n. sp. and *G. ozimeci* n. sp. To analyze interspecific morphological differences within *Graciliella* we performed a discriminant analysis based on 40 linear morphometric measurements. The results showed that differences between species and subspecies inside *Graciliella*, however subtle they may seem, are measurable and reproducible. All species of the genus are briefly diagnosed, an identification key is proposed and a distribution map of all taxa of *Graciliella* is provided.

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Introduction

Unrelated organisms that are placed under similar, severe selection pressures often converge on (or evolutionarily remain trapped in) a limited set of body shapes, and may therefore be hard to classify morphologically. Extreme environments (*e.g.* subterranean habitats, deep sea, polar regions, deserts) exert strong directional selection upon their inhabitants, leading to convergent and generally predictable outcomes (Cloudsley-Thompson, 1988; Trontelj *et al.*, 2012). As a consequence, extremophiles found in Arctic tundra (Grundt *et al.*, 2006), subterranean habitats (Lefébure *et al.*, 2006) and deep-sea environments (Vrijenhoek *et al.*, 1994) are well-known for their morphological uniformity and cryptic diversity.

Subterranean environments are considered to be extreme because of highly stable abiotic conditions and low nutrient availability. Species that have colonized these habitats evolve a somewhat predictable suite of morphological, physiological and behavioural characteristics (Gross, 2012; Trontelj *et al.*, 2012), including eye degeneration, depigmentation, development of sensory organs, longer life cycles, lower metabolic rate,

and body shape modifications (Racovitza, 1907; Vandel, 1964; Culver *et al.*, 1990; Faille *et al.*, 2009). This set of characteristics is known as ‘troglobiomorphy’ or ‘troglomorphy’ and organisms characterized by these features are often restricted to subterranean environment (trogllobites).

The troglomorphic phenotype shared by diverse cave-adapted animals is often regarded as a classical example of convergent evolution (Stemmer, 2015). Many studies have shown troglomorphic convergence in different taxa (Christiansen, 1961; Hedin and Thomas, 2010; Protas *et al.*, 2006), which may complicate phylogenetic reconstruction by morphological approaches. Molecular techniques have become a crucial tool to overcome this problem and to efficiently reveal phylogenetic relationships among different taxonomic categories. They have been recently involved in elucidating the phylogeny of different groups with subterranean representatives such as Bivalvia (Stepien *et al.*, 2001), Dytiscidae (Leys *et al.*, 2003), Decapoda (Zakšek *et al.*, 2007), Parabathynellidae (Abrams *et al.*, 2012), Trechini (Faille *et al.*, 2009; 2013) and Leptodirini (Caccone and Sbordonì, 2001; Ribera *et al.*, 2010; Fresneda *et al.*, 2011; Cieslak *et al.*, 2014a; 2014b). Molecular techniques have helped in distinguishing morphological characters inherited from a common ancestor from those resulting from recent, independent adaptation to a specific biotope.

Among Insecta, Coleoptera are the dominant group that have colonized subterranean habitats (Sket, 2005). Two groups of Coleoptera are particularly diverse in caves: the subfamily Cholevinae in the suborder Polyphaga, and the subfamily Trechinae in the suborder Adephaga (Casale *et al.*, 1998; Faille *et al.*, 2009). With more than 230 genera and 900 (mostly polytypic) species, the tribe Leptodirini (Cholevinae) forms one of the most species-rich tribes of cave-adapted Coleoptera, surpassed only by Trechini (Carabidae). Most are subterranean, living in caves and fissures, except a few species inhabiting underground termite nests or superficial litter layers. Within the Leptodirini, four morphological types are recognized: bathyscioid, pholeuonoid, scaphoid, and leptodiroid (Jeannel, 1924). The leptodiroid morphological type, named after *Leptodirus hochenwartii* Schmidt, 1832 from Slovenia, Croatia, and Italy, is the most derived morphologically, compared to the presumed ancestral body plan (Sket, 2005). Species belonging to this morphological type have extremely long appendages, extremely elongated head and pronotum, hemispherical elytra, and are fully anophthalmic. The subtribe Anthroherponina com-

prises taxa of exclusively leptodiroid morphological type, showing the most pronounced troglomorphic characters among Leptodirini (Njunjić *et al.*, 2015). To date, taxonomic studies of Anthroherponina were based exclusively on morphological traits, but possible phenotypic convergence leaves these conclusions doubtful.

To test if characters that have traditionally been accepted as indicative of Anthroherponina classification are indeed reliable, we evaluated the monophyly of the most species-rich genus of this subtribe – *Anthroherpon* Reitter, 1889. Taxonomy of this genus has always been difficult. Most taxa were described in the late 19th century and in the second half of the 20th century, on a small number of specimens and without an explicit diagnosis, so many were subsequently synonymized. Moreover, the geographical distribution of some taxa is mysteriously disjunct (Njunjić *et al.*, 2015).

The present work provides a first phylogenetic analysis of three closely related genera of the subtribe Anthroherponina: *Anthroherpon*, *Leptomeson* Jeannel, 1924, and *Hadesia* Müller, 1911, combining morphological and molecular approaches. We clarify their generic status and we reveal the existence of the fourth, new genus, *Graciliella*, for which we then examine the intrageneric diversity. Additionally, two new species belonging to this new genus are described.

Material and methods

Acronyms

The following abbreviations for collections and institutions are used: CNHM: Croatian Natural History Museum, Zagreb, Croatia; MNHN: Muséum National d'Histoire Naturelle, Paris, France; Naturalis: Naturalis Biodiversity Center, Leiden, The Netherlands; NMP: Narodni Museum, Prague, Czech Republic; NHM: Naturhistorisches Museum Wien; CINJ: Iva Njunjić Collection, Belgrade, Serbia; CMPR: Michel Perreau Collection, Paris, France; CDPV: Dragan Pavićević Collection, Belgrade, Serbia.

The following abbreviations for morphological details are used: HL: head length (measured from the posterior margin of the clypeus); HW: maximum width of the head; PL: pronotum length (measured along the median line); Pwmax: maximum width of the pronotum (largest transverse width); Pwmin: minimum width of the pronotum (shortest transverse width), PL/Pwmax: length of the pronotum divided by maximum

width of the pronotum; MPI: mesothoracic pedunculus length, MPwmin: minimal width of the mesothoracic pedunculus; PL/MPI: length of the pronotum divided by the length of mesothoracic pedunculus; EL: elytra length (as linear distance measured along the median line from the base to the apex); EW: maximum width of the elytra; EL/EW: length of the elytra divided by maximum width of the elytra; MP2/MP3: length of the second maxillary palp divided by the length of the third maxillary palp. Reference to type material is abbreviated as HT: holotype.

Taxon sampling and morphological study

Specimens were collected in caves of the Dinaric range, in Montenegro, Bosnia and Herzegovina, and Croatia as listed in S1. For amplification and sequencing we used two specimens per population of the genus *Graciliella* n. gen. and *Anthroherpon*, and one specimen per population of the genus *Hadesia* Müller, 1911. Since specimens from the same population had almost identical 28S, 18S and COI gene fragments, we used only one individual per population for the phylogenetic tree. A total of 44 specimens representing 27 taxa from three genera (*Anthroherpon*, *Hadesia*, and *Leptomeson*) were included in the molecular analysis. We chose these genera because they are the most species-rich Anthroherponina and because major taxonomic issues

need to be resolved among them. As outgroups we chose several subterranean species or subspecies from other genera of the tribe Leptodirini: *Parapropus sericeus muelleri* Jeannel, 1924, *Charonites* Apfelbeck, 1907 sp., and *Apholeuonus nudus sturanyi* Apfelbeck, 1906 of which we sequenced a single individual per population. To root the tree, we used *Speonesiotes* Jeannel, 1910 sp. belonging to the subtribe Bathysciotina. In total we sampled 36 specimens of 18 species of *Anthroherpon*, 4 specimens of 4 species of *Hadesia*, and 4 specimens of 4 species of *Leptomeson* (Table 1; S1).

After extraction, male genitalia were cleared in clove oil, mounted in Euparal on glass slides and pinned beneath the specimens. The external morphology of specimens was examined using Leica MZ 75 and Leica M10 stereomicroscopes. Microphotographs were taken on a Leica DIAPLAN or a Zeiss AXIO-LAB microscope with a camera diagnostic instrument Spot INSIGHT IN1820. Macrophotographs were taken using a Canon Eos 70D camera with Canon EF MP-E 65mm f/2.8 macro lens and Canon macro twin lite mt-24ex flash. Digital pictures were processed using the Helicon Focus software 5.3 (<http://www.heliconsoft.com/heliconsoft-products/helicon-focus/>).

DNA extraction, PCR amplification, and sequencing

The specimens used in the study were collected alive in the field and preserved in 96% ethanol. DNA was extracted from whole specimens or from one leg with a standard phenol-chloroform extraction (Blin and Stafford, 1976) or the DNeasy Tissue Kit (Qiagen GmbH, Hilden, Germany). Voucher specimens are stored in the MNHN (Paris, France), CNHM (Zagreb, Croatia) and DNA aliquots are kept in the tissue collections of Naturalis (Leiden, Netherlands).

We amplified fragments of two nuclear genes: 5' end of the small ribosomal unit, 18S rRNA (SSU) and

Table 1. Genera with total number of species and number of species included in the study. Taxonomy follows Perreau (2004), updated.

Genus	N. spp.	Sampled spp.
<i>Anthroherpon</i>	30	18
<i>Hadesia</i>	4	4
<i>Leptomeson</i>	9	4

Table 2. Primers used in the study.

Fragment	Name	Sense	Sequence 5'-3'	Reference
<i>COIa</i>	LCOI-1490	F	GGTCAACAAATCATAAAGATATTG	Folmer <i>et al.</i> (1994)
<i>COIa</i>	HCOI-2198	R	TAAACTTCAGGGTGACCAAAAAATCA	Folmer <i>et al.</i> (1994)
<i>COIb</i>	Jerry	F	CAACATTTATTTTGATTTTGG	Simon <i>et al.</i> (1994)
<i>COIb</i>	Pat	R	TCCAATGCACTAATCTGCCATATTA	Simon <i>et al.</i> (1994)
<i>18S</i>	5'	F	GACAACCTGGTTGATCCTGCCAGT	Shull <i>et al.</i> (2001)
<i>18S</i>	b5.0	R	TAACCGCAACAACCTTAAT	Shull <i>et al.</i> (2001)
<i>28S</i>	Ka	F	ACACGGACCAAGGAGTCTAGCATG	Ribera <i>et al.</i> (2010)
<i>28S</i>	Kb	R	CGTCCTGCTGTCTTAAGTTAC	Ribera <i>et al.</i> (2010)

an internal fragment of the large ribosomal unit, 28S rRNA (LSU), and of two non-overlapping sections of mitochondrial gene fragments—the 5' and 3' halves of cytochrome c oxidase subunit 1 (which we here term COIa and COIb, respectively). Primers used are given in Table 2, and PCR protocols are given in S2. Sequences were assembled and edited using Geneious version 8.0.5 (<http://www.geneious.com/>, Kearse *et al.*, 2012). DNA sequences obtained for each genetic marker were aligned separately using MAFFT version 7 (Kato and Standley, 2013). Sequences have been deposited in GenBank with Acc. Nos (S1).

Phylogenetic analyses

For each sequence alignment, the optimal model of nucleotide substitution was determined using jModel-Test2 (Darriba *et al.*, 2012; Guindon and Gascuel, 2003), run on the CIPRES webportal (Miller *et al.*, 2010). Selection was based on the Akaike Information Criterion (AIC), and resulted in selection of TIM2ef+I, GTR+G, TPM1uf+I+G and TPM3uf+I+G substitution models for 18S, 28S, COIa and COIb, respectively. We

did two separate phylogenetic analyses - one at the genus level, using all three loci, and one at the species level within *Graciliella* using only COI. Phylogenetic analysis was performed using MrBayes 3.2.2 (Ronquist and Huelsenbeck, 2003) on CIPRES (Miller *et al.*, 2010), with settings for multiple markers unlinked, running two replicates of 15×10^6 generations each. Sample frequency was set to 5000. Convergence diagnostics were run using Tracer version 1.5 (Rambaut *et al.*, 2014), where ESS values for all parameters were $>>200$. After discarding a 25% burn-in, the resulting majority-rule consensus tree was visualized using FigTree version 1.4 (Rambaut, 2012).

Morphometric analysis

We subjected a total of 41 individuals, all males, belonging to *G. apfelbecki apfelbecki* (14 specimens), *G. apfelbecki scutulatum* (1 specimen), *G. metohiensis* (14 specimens), *G. absoloni* (1 specimen), *G. lahneri* (2 specimens), *G. ozimeci* (9 specimens), and *G. kosovaci* (1 specimen) to linear morphometric measurement with a Micro-Vu Vertex 251HC ([The figure is a Bayesian inference phylogenetic tree. The root is at the top left, with *Speonesiotes* sp. \[IO39\] as the outgroup. The tree branches downwards and to the right. The main clade includes *Parapropus sericeus muelleri* \[IO26\], *Apholeuonus nudus styranii* \[IAP3\], and a large clade of *Hadesia* species. The *Hadesia* clade includes *H. asamo* \[MP01\], *H. vasiccki* \[IH1\], *H. lakotai* \[MP03\], *H. weiratheri* \[IH2\], *Anthroherpon cylindricolle cylindricolle* \[IE3\], *Anthroherpon pygmaeum pygmaeum* \[IE25\], *Anthroherpon charon* \[VA34\], *Anthroherpon harbichi* \[IB3\], *Anthroherpon latipenne* \[IE13\], *Anthroherpon taxi taxi* \[IE55\], *Anthroherpon weiratheri* \[IE21\], *Anthroherpon stenocephalum stenocephalum* \[IE44\], *Anthroherpon zariquieyi* \[IE37\], *Anthroherpon hoermanni hoermanni* \[IB7\], *Anthroherpon primitivum primitivum* \[IE39\], *Anthroherpon ganglbaueri ganglbaueri* \[IE11\], *Anthroherpon sinjajevina* \[VA6\], *Anthroherpon matzenaueri* \[IB13\], and *Anthroherpon matulici* \[I5\]. Below this is a clade of *Leptomeson* species: *L. radjai* \[IL1\], *L. bujasi* \[IL4\], *L. dombrowskii dombrowskii* \[IL2\], and *L. raguzi* \[IL3\]. At the bottom is a clade of *Graciliella* species: *G. metohijensis* \[IB15\], *G. kosovaci* sp.n. \[IE63\], *G. apfelbecki apfelbecki* \[VA43\], and *G. ozimeci* sp.n. \[VA44\]. Posterior probabilities are shown above the nodes. A scale bar of 0.02 is provided at the bottom left.](https://www.</p>
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Fig 1. Bayesian inference of combined 18S, 28S, COIa, and COIb data sets for *Hadesia*, *Anthroherpon*, *Leptomeson*, and *Graciliella*. Numbers above nodes are posterior probabilities.

microvu.com/), three-dimensional set-up, using Inspec Metrology Software (<https://www.inspec-inc.com/>). We used 40 landmarks on antennae, maxillary palps, head, thorax, abdomen, and legs. Each individual was measured three times. In a small number of cases, obvious measurement errors were detected a posteriori (values differing by a factor of >2 from the other two replicates of the same individual). These were then replaced by the average value of the other two replicates. The full list of measured material and their respective morphometrics is given in S3 and S5; the derivations of the 40 linear measurements are given in S4. We first checked, for those species for which we had DNA extracts, whether the species that had been recognized by traditional, qualitative morphological study, could also be recovered as monophyletic groups in the molecular phylogeny (see below). As this appeared to be the case, we then subjected this set of independently obtained quantitative measurements to a Discriminant Analysis in PAST 3.10 (Hammer *et al.*, 2001), to investigate whether a more objective morphometric analysis would allow us to recognize the same groups.

Results and Discussion

Our study, based on morphological and molecular approaches, reveals that the genus *Anthroherpon* as conventionally defined (Jeannel, 1924; Guéorguiev 1990; Giachino and Gueorguiev, 1993; Giachino and Vailati, 2005; Perreau and Pavićević, 2008) is polyphyletic (Fig. 1). The genus *Leptomeson* forms the highly supported sister clade of one polytypic *Anthroherpon* species, namely *A. apfelbecki* sensu lato and the genus *Hadesia* forms the sister clade of the remaining *Anthroherpon* species. To resolve this polyphyly, we defined one new additional genus, *Graciliella*, comprising *A. apfelbecki* sensu lato and one species, *A. absoloni* (Guéorguiev, 1990), for which we did not have genetic data, but which morphologically closely resembles *A. apfelbecki* (see below under the genus description). Morphological phylogenetic analysis of the species of *Hadesia* was obtained by Perreau and Pavićević (2008). Their parsimony analysis gave a well-supported monophyletic group for *H. vasiceki* Müller, 1911 and *H. lakotai* Perreau and Pavićević, 2008 (bootstrap value 100 %), but the possible sister group *Hadesia asamo* Perreau and Pavićević, 2008 + *H. weiratheri* Zariquiey, 1927 was weakly supported (bootstrap value 50 %). However, analysis based on distances as optimality criterion supported both

groups: *H. vasiceki* + *H. lakotai* and *H. asamo* + *H. weiratheri* as sister groups at the same level of bootstrap value (Perreau and Pavićević, 2008). Our phylogenetic tree based on molecular data has a different topology – *H. asamo* and *H. vasiceki* are early-branching and *H. lakotai* and *H. weiratheri* are forming a clade. These results are surprising regarding the distribution of the species. Namely, *H. asamo* and *H. weiratheri* are both located in the Orjen massif in Montenegro while *H. weiratheri* and *H. lakotai* were found in remote massifs, in caves which are more than 50 km apart. Moreover, *H. asamo* and *H. weiratheri* are morphologically extremely similar (Perreau and Pavićević, 2008) which can easily mislead phylogenetic reconstruction by morphological approaches.

The genus *Leptomeson* was originally established as a subgenus of *Anthroherpon* (Jeannel, 1924) and then subsequently raised to genus rank by Guéorguiev (1990). This separation was based on the following characters of *Leptomeson*: more developed mesothoracic pedunculus, with constriction in the middle part and wider in the base, endophallus with differentiated basal ‘armature’ (sclerified stylet-shaped phanera, Giachino *et al.*, 2011) and parameres bearing 4 setae. Further investigations and discoveries of several new species belonging to this genus have shown that number of setae on parameres varies in fact from 3 to 4 (Giachino *et al.*, 2011). The molecular phylogenetic reconstruction is in accordance with the morphology regarding the separation of the genus *Leptomeson* from *Anthroherpon*. The clade *Graciliella* + *Leptomeson*, in addition to being clearly supported by the molecular analysis, is also morphologically supported by the following characters that are likely synapomorphies: very elongated pronotum and mesothoracic pedunculus, and mesoventrum without processus between mesocoxae. Mesoventral processus is normally entire (it completely separates the two mesocoxal cavities) in Leptodirini, except in some highly evolved groups such as Anthroherponina in which it is reduced or absent (Jeannel, 1911). We observed that the degree of reduction of this processus is a distinguishing character among Anthroherponina: it is present in the genus *Anthroherpon*, and absent in *Leptomeson* and *Graciliella*. However, this character is still insufficiently explored in other Anthroherponina to assess its relevance in non-Anthroherponine Leptodirini. For instance, *Anthroherpon stenocephalum* (Apfelbeck, 1910) is the only species of the genus *Anthroherpon* in which we noticed intrapopulation variability of this character: some specimens have a mesoventral processus between

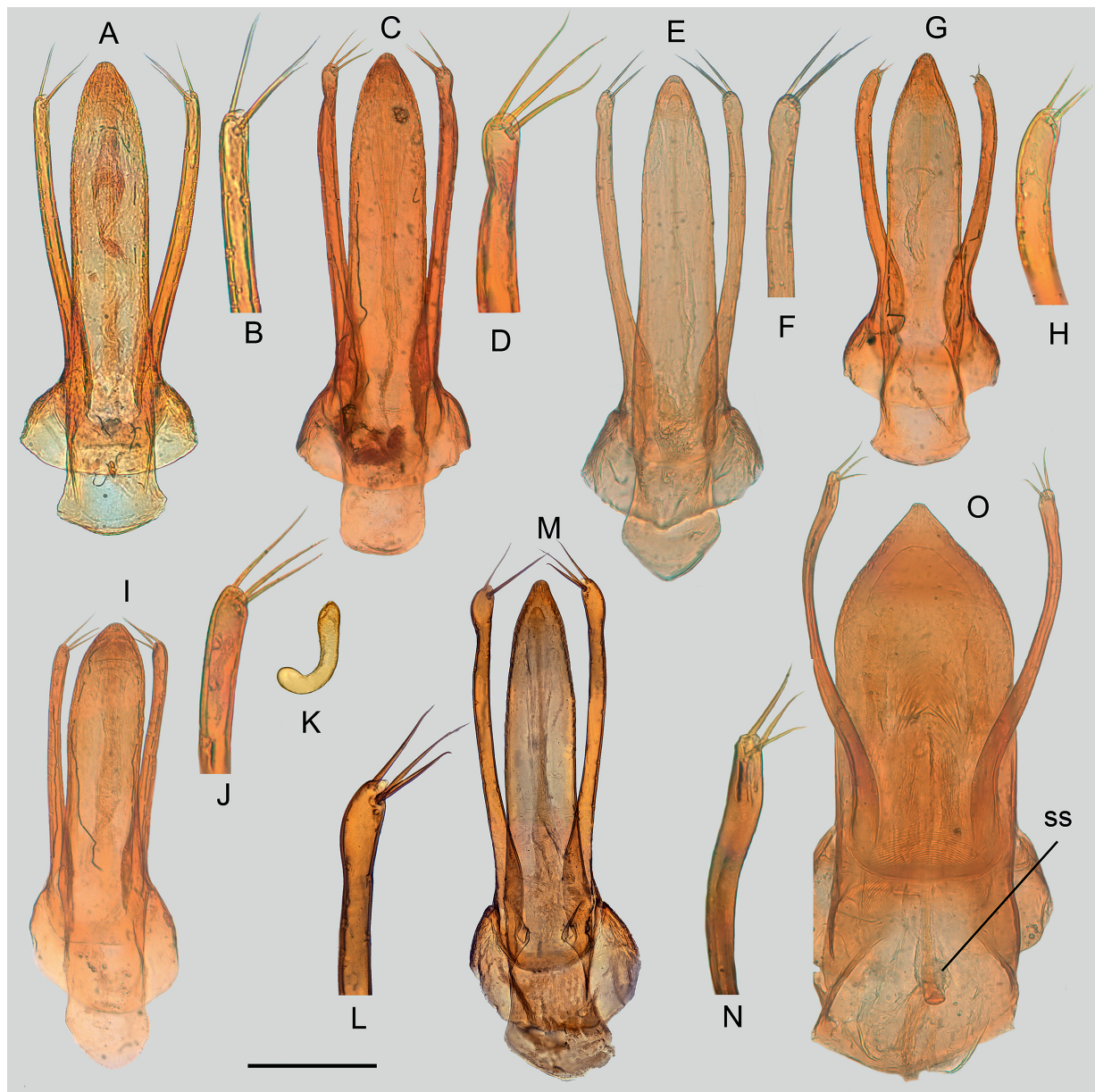


Fig. 2. Genital structures of *Graciliella* and *Leptomeson*, complete aedeagus + detail of paramere apex: A-B. *G. apfelbecki apfelbecki*; C-D. *G. metohijensis*; E-F. *G. absoloni*; G-H. *G. kosovaci* sp. n.; I-J. *G. ozimeci* sp. n.; L-M. *G. lahneri*; N-O. *L. leonhardi*; ss. sclerified structure in the endophallus; K. spermatheca of *G. ozimeci* sp. n. Scale bar is 0.1 mm

the mesocoxae while it is absent in others, but no intermediate state was observed. The monophyly of the genus *Leptomeson* is morphologically supported by the presence of the sclerotized structures in the endophallus (Fig. 2O), which are absent in *Graciliella*, and the shape of mesothoracic pedunculus with the constriction in the mid-section or posterior of the mid-section (Fig. 3C).

A dorsal comb of very short bristles on the terminal maxillary palpomere is present in three genera that we analyzed in this study (*Anthroherpon*, *Leptomeson*, and *Graciliella*). However, it is absent in *Hadesia*. We could find no previous literature regarding this character so that we believe it was not previously observed. Given the scarcity of nutrients in the subterranean

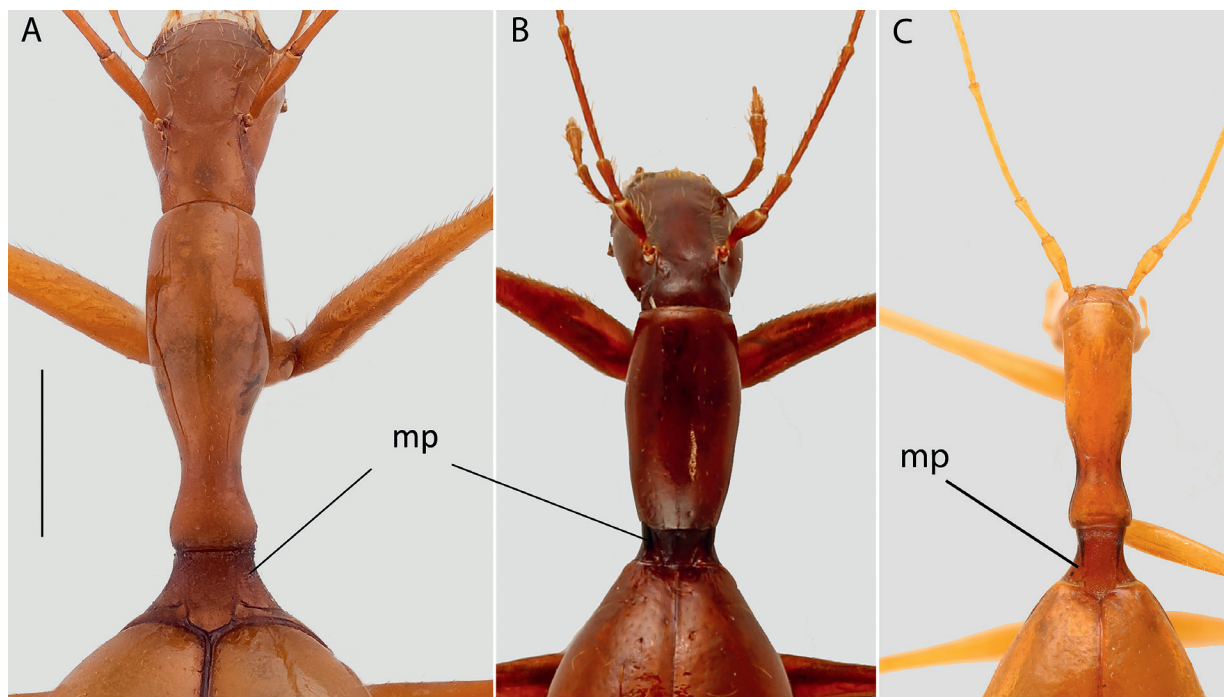


Fig. 3. Pronotum and mesothoracic pedunculus: A. *Graciliella metohijensis*; B. *Anthroherpon cylindricolle cylindricolle*; C. *Leptomeson bujasi*; mp, mesothoracic pedunculus. Scale bar is 1 mm.

environment, this comb is possibly an extra sensory organ that has a yet to be determined role in detecting food.

Our molecular analysis shows that the four *Graciliella* species are genetically well-separated, with COI-differences between 3.9 and 12.4%, values that are normally found at or above the species level in Cholevinae (Schilthuizen *et al.*, 2011) (Fig. 4). To confirm that our mostly genetically circumscribed species indeed correspond with morphologically recognizable units, we carried out a linear discriminant analysis (LDA) based on 40 linear morphometric measurements. An LDA is especially suited for maximizing inter-group separation and minimizing intra-group separation for pre-defined units (McLachlan, 2004). This LDA confirmed that the interspecific morphological differences, however subtle and minute as they may seem, are measurable and reproducible. Along the first two axes (with major loadings for tibia lengths, lengths of antennomeres 4 and 11, and elytra length), all species can be distinguished, except the two new species, *G. kosovaci* and *G. ozimeci* (Fig. 5A). These latter two species, however, are well-separated along the third discriminant axis, in which there are additional loadings for the first meso- and metatarsomere

(Fig. 5B). Based on this independent, initial morphometric separation, we then detected additional qualitative diagnostic characters in setation, shape of pronotum, aedeagus, and microsculpture. The diagnoses and identification keys are largely based on these qualitative characters, as they are more easily accessible.

Perreau and Pavićević (2008) reconstructed the phylogeny of the subtribe Anthroherponina based on 11 morphological characters. Their most parsimonious tree shows *Leptomeson* and *Anthroherpon* as sister genera, and *Hadesia* distantly related, as sister genus of *Kircheria* Giachino and Vailati (2006). The character states that Perreau and Pavićević defined for *Anthroherpon* also apply to all species of *Graciliella*. If we rather accept our molecular phylogeny, in which *Graciliella* is a sister clade of *Leptomeson*, and *Anthroherpon* s.str. sister clade of *Hadesia*, this would imply that a large number of morphological characters are strongly homoplasious. These characters are: female ventrite VIII with an anterior apophysis versus without, aedeagus with internal sclerified structure versus without, abdominal ventrites with glabrous plates versus without glabrous plates, female first ventrite with lateral hollows versus without, third maxillary palpomere short versus long, claws narrow versus wide. Although

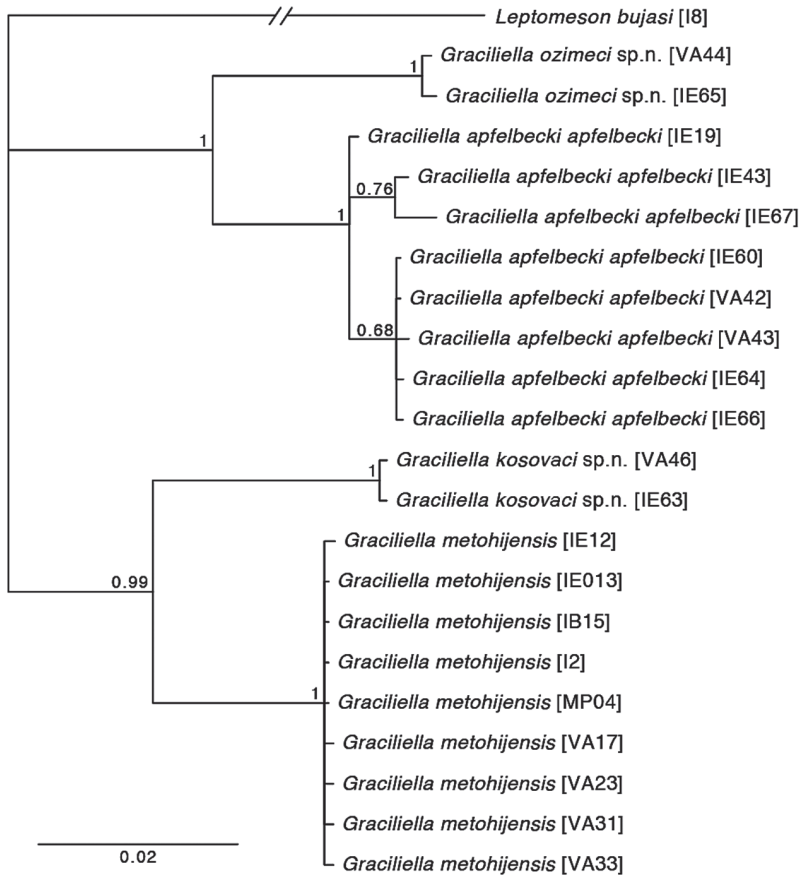


Fig. 4. Bayesian inference of combined COIa and COIb data sets for *Graciliella*. Numbers above nodes are posterior probabilities.

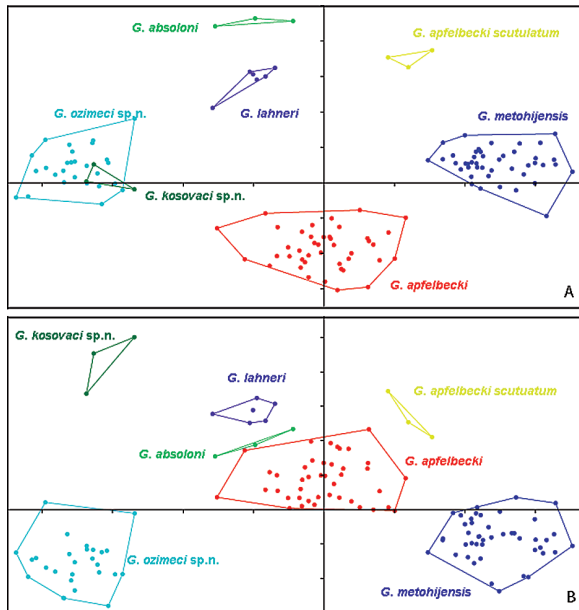


Fig. 5. Linear discriminant analysis (LDA) based on 40 linear morphometric measurements. A. Axes 1 and 2; B. Axes 1 and 3.

many of these characters seem reliable and have traditionally been accepted as indicative of *Anthroherponina* classification, our results suggest that perhaps this could be attributed to convergence due to shared selective pressures rather than to shared ancestry.

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Online supplementary information

S1. Sequenced specimens, with depository, locality, collectors, and sequence accession numbers.

S2. PCR cycling conditions.

S3. The list of material included in the morphometric analysis (Sheet 1) and list of measured traits (Sheet 2).

S4. The derivations of the 40 linear measurements.

S5. Landmarks recorded on the body of *Graciliella* spp.

Appendix

Systematic part

Above, where we discuss the results of molecular phylogenetics, we show that the genus *Anthroherpon* is polyphyletic. For this reason we erect a new genus, *Graciliella*. In this systematic section, we describe and diagnose this genus and its constituent species. For full treatment of molecular phylogenetics we refer to the Results section.

<http://zoobank.org/urn:lsid:zoobank.org:pub:0A490485-E40F-4422-8D34-D78A92716FB0>

Graciliella n. gen.

urn:lsid:zoobank.org:act:B0FA4AC2-76D4-4208-8053-317D44E61E42

Type species: *Graciliella apfelbecki apfelbecki* (Müller, 1910)

Description. With a body length from 7.09 to 8.63 mm (HT 8.33 mm), this new genus is one of the largest-bodied Leptodirini known from the Dinaric Mountains. General morphology leptodiroid; pronotum and mesothoracic pedunculus very elongated, elytra physogastric; eyeless, wingless, and depigmented.

Head. Elongated and hypognathous, wider in the anterior part and wider than the pronotum, HL 1.33–1.58 mm (HT 1.55 mm), HW 0.75–0.93 mm (HT 0.83 mm). Dorsal surface shiny or matte, with superficial microreticulation visible at 40 x magnification under a stereomicroscope and with sparse and short hairs. Few random punctures present on the dorsal surface of the head and most numerous on the frons. Dorsal side of the occiput bearing two patches of punctures with very short hairs on both sides of the head. Labrum trapezoid with setae longer than on the frons. Maxillae long, both galea and lacinia elongated and with brushes of setae. Maxillary palps with 4 articles; second maxillary palpomere arched and about the same length as the third one; terminal maxillary palpomere is the shortest and conical in shape; mean MP2/MP3: 1.33. The base of the terminal maxillary palpomere with a dorsal comb of very short bristles (Fig. 6). Labium with trapezoid submentum bearing two long setae. Mandibles angulate in dorsal view, with sharp apex and with 3 or 4 small teeth between the apex and the last big tooth. Antennal insertions are located in the posterior quarter of the length of the head. Antennae

longer than the body, second antennomere is the shortest and last one is longer than the penultimate in both sexes. All antennomeres are covered with hairs of medium length, but only antennomeres 7–11 bear up to 8 long, erected setae arranged circularly and symmetrically in apical part (Fig. 7A). Three last antennomeres (9, 10, 11) bear very short and thin hairs inserted between the hairs of medium length and long apical setae. These short, thin hairs are the most numerous on the last antennomere where they form dense covering (Fig. 7B).

Pronotum. Distinctly longer than wide, narrow, and with a strong constriction in the posterior quarter. Maximal width is in the second third of its length; Pwmax: 0.6–0.8 mm (HT 0.72 mm); Pwmin: 0.3–0.45 mm (HT 0.36); PL: 1.77–2.27 mm (HT 2.18). Sides divergent in the anterior part of the pronotum, before its maximal width and convergent in the posterior quarter of the length of the pronotum. Surface shiny or matte, with evident microreticulation visible at 40 x magnification under a stereomicroscope. Without or with few very short hairs, mostly in the posterior part.

Mesothoracic pedunculus. Elongated, PL/MPI: 4–5.3 mm (HT 5.06); slightly wider (minimal width) than long or as long as wide; minimal width in the anterior part; MPI: 0.31–0.48 mm (HT 0.43), MPwmin: 0.42–0.55 mm (HT 0.51). Ecusson (scutum+scutellum; Jeannel, 1911) rough, ‘U’-shaped and with microsculptural mesh resembling honeycomb pattern.

Elytra. Elliptic, physogastric, and wide; maximum width in the anterior part of the mid-section, at the mid-section, or slightly posterior of the mid-section, EL: 3.48–4.54 mm (HT 4.16), EW: 2.19–3.42 (HT 2.78). Surface smooth, shiny or matte, generally with no microreticulation except in some specimens of *Graciliella ozimeci* sp. n. Punctuation superficial, spaced, and irregular; if present, hairs are sparse, short or long.

Abdomen. Transversal carina on the posterior margin of mesoventrum non-interrupted, without mesoventral process; mesocoxal cavities widely confluent (Fig. 8B). Abdominal ventrites densely covered with short hairs except in the anterior part where they are smooth.

Legs. Long, slender, and pubescent. Femora enlarged in the basal parts, tibiae straight or slightly curved inwards (protibiae). Male protarsi with five protarsomeres, female protarsi with four protarsomeres, mesotarsi and metatarsi with five tarsomeres in both sexes. Tarsi not dilated, tarsal empodium with two long setae.

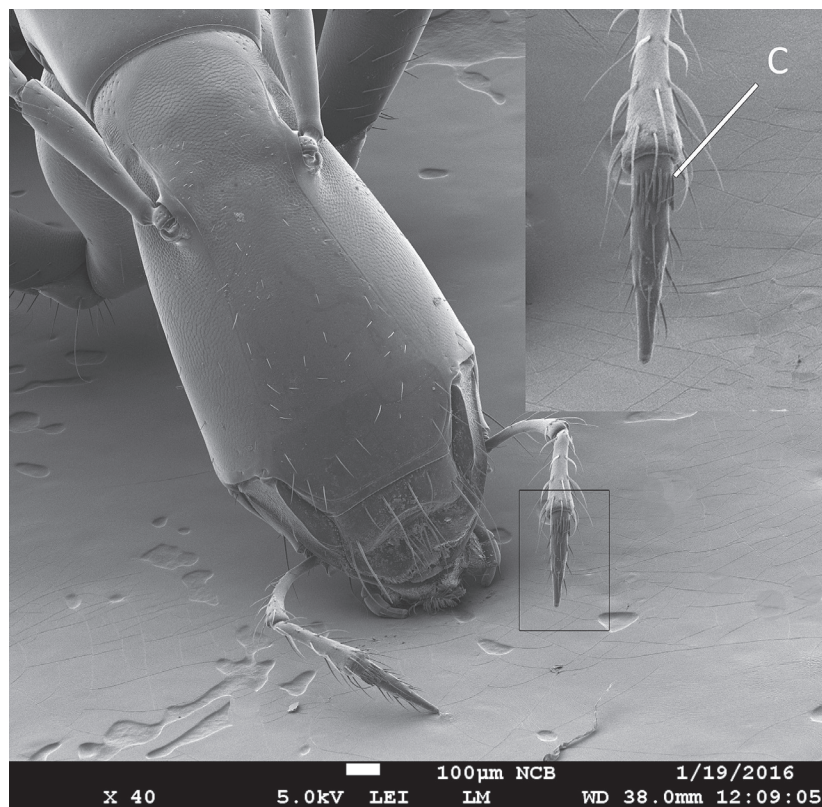


Fig. 6. Terminal maxillary palpomere with a dorsal comb of bristles (c).

Aedeagus (Fig. 2A–J, L, M). Slender, median lobe with rounded, lanceolate or acute apex, endophallus without ‘sclerified stylet-shaped phanera in the median part’ (Giachino *et al.*, 2011) *i.e.*, endophallus without sclerotized structures. Parameres as long as or shorter than the median lobe (setae excluded), bearing three apical setae. Urite IX reduced to a ring, as in most Leptodirini.

Female genitalia. Abdominal ventrite VIII with a long and narrow anterior expansion. Appendicular parts (gonocoxites and gonosubcoxites, cf. Deuve, 2001 for terminology) of the urite IX absent. Spermatheca: weakly sclerified, ‘J’-shaped, short and slightly widened in the apical region (Fig. 2K).

The genus comprises the following taxa that were previously placed in ‘*hoermanni*’ species group of the genus *Anthroherpon*:

Graciliella absoloni (Guéorguiev, 1990) n. comb.

Graciliella apfelbecki apfelbecki (Müller, 1910) n. comb.

Graciliella apfelbecki scutulatum (Giachino and Guéorguiev, 1993) n. comb.

Graciliella apfelbecki schwienbacheri (Giachino and Vailati, 2005) n. comb.

Graciliella apfelbecki sculptifrons (Winkler, 1925) n. comb.

Graciliella lahneri (Matcha, 1916) n. comb., n. stat

Graciliella metohijensis (Zariquiey, 1927) n. comb., n. stat

Graciliella kosovaci sp. n.

Graciliella ozimeci sp. n.

After removing these taxa, the ‘*hoermanni*’ species group comprises: *Anthroherpon hoermanni hoermanni* (Apfelbeck, 1889), *A. hoermanni hoffmanni* Giachino and Guéorguiev, 1993, *A. hoermanni hypsophilum* Apfelbeck, 1907, *A. hoermanni orlovacensis*, Guéorguiev, 1990, *A. hoermanni sericeum* Jeannel, 1930, *A. scutariensis* Giachino and Guéorguiev, 1993.

Cross diagnosis. The new genus can be easily distinguished from the genus *Anthroherpon*, even with the naked eye, by the very elongated mesothoracic pedunculus, narrowest in the anterior part, shape of pronotum (Fig. 3) and more spherical-shaped elytra. Additional difference is in the mesoventral processus which

is absent in *Graciliella* and present in *Anthroherpon* (Fig. 8). It differs from the genus *Leptomeson* by the following combination of characters: larger body size (from 7.09 to 8.63 mm in *Graciliella* and from 4.63 to 7.22 mm in *Leptomeson*), shape of mesothoracic pedunculus and elongated male genitalia without sclerotized structures in the endophallus (Fig. 2A-J, L, M). In *Graciliella*, the narrowest part of the mesothoracic pedunculus is in the anterior part while in *Leptomeson* it is in the mid-section or posterior of the mid-section (Fig. 3).

Distribution. The distribution areas are shown on the map of Fig. 9.

Etymology. The new generic name derives from the Latin word '*gracilis*' which means slender and refers to the habitus of the genus.

Identification key to separate genera

The following identification key of the genera of the subtribe Anthroherponina modifies and updates the respective section of the key given by Perreau and Pavićević (2008):

8. Pronotum and mesothoracic pedunculus less elongated. Mesothoracic pedunculus short or absent; PL/MPI > 5.3 mm (Fig. 3B). Transversal carina on the posterior margin of mesoventrum interrupted, mesoventrum with long processus between mesocoxae (there is an exception; see: Discussion) (Fig. 8A) *Anthroherpon* Reitter
- Pronotum and mesothoracic pedunculus very elongated. Mesothoracic pedunculus long, always

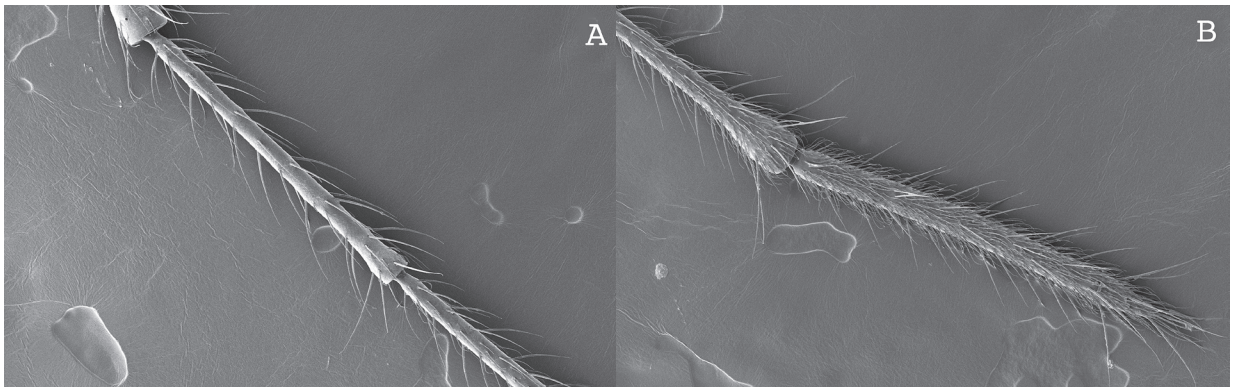


Fig. 7. Antennomeres: A. antennomeres 7 and 8; B. antennomeres 10 and 11.

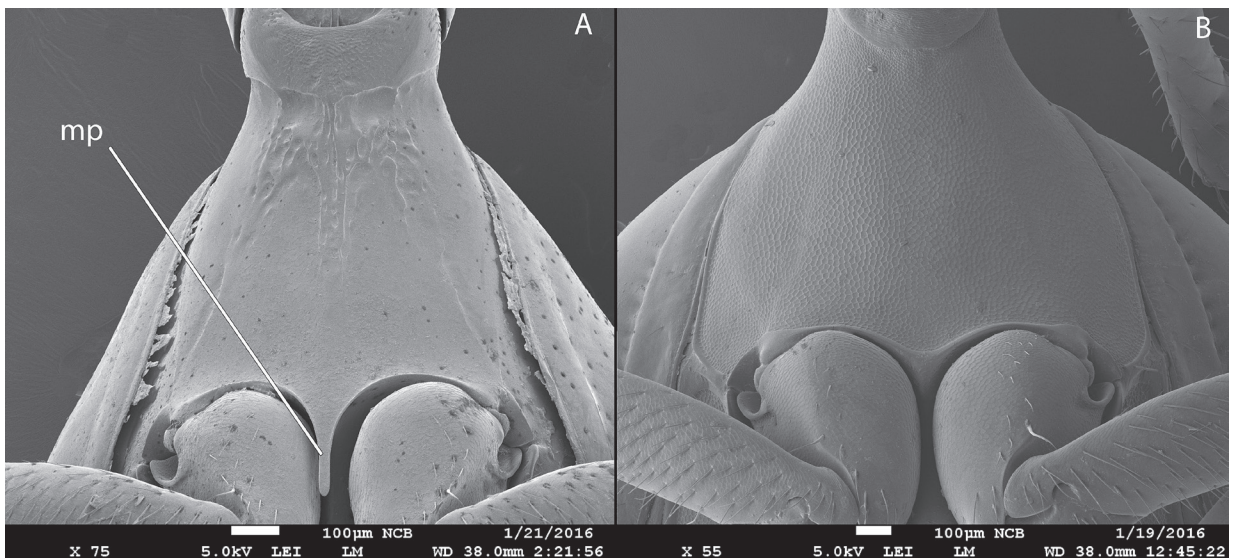


Fig. 8. Mesoventrum of *Anthroherpon* and *Graciliella*: A. *Anthroherpon cylindricolle cylindricolle*, B. *Graciliella metohijensis*; mp. mesoventral processus.

- present; PL/MPI < 5.3 mm. Transversal carina on the posterior margin of mesoventrum non-interrupted, mesoventrum without processus between mesocoxae 9
9. Mesothoracic pedunculus narrowest in the anterior part (Fig. 3A). Aedeagus slender, without sclerotized structures in the endophallus, parameres bearing 3 setae, (Fig. 2A-J, L, M), body size from 7.09 to 8.63 mm *Graciliella* n. gen.
- Mesothoracic pedunculus narrowest in the mid-section or posterior of the mid-section (Fig. 3C). Aedeagus robust, with sclerotized structures in the endophallus, parameres bearing 3 or 4 setae, (Fig. 2N,O), body size from 4.63 to 7.22 mm *Leptomeson* Jeannel

***Graciliella kosovaci* sp. n.**

urn:lsid:zoobank.org:act:CCB91AA9-E5D4-4DCE-9070-C5A3A89C2BFB

Type series. Holotype male labeled as follows: ‘CROATIA, Popovići, Gruda, Konavle, Kaverna 781, 50 m, 09.11.2014, leg. R. Ozimec’ (white label, printed)/ ‘HOLOTYPUS *Graciliella kosovaci* sp. n. I. Njunjić, M. Perreau, K. Hendriks, M. Schilthuizen and L. Deharveng det. 2015’ (red label, printed), (CNHM). Paratype: 1♀, 09.11.2014, same cave, leg. R. Ozimec,

(CNHM). Paratype is labeled with white, printed locality labels and with red printed labels ‘PARATYPUS *Graciliella kosovaci* sp. n. I. Njunjić, M. Perreau, K. Hendriks, M. Schilthuizen and L. Deharveng det. 2015’.

Type locality. Croatia, Popovići, Gruda, Konavle, Kaverna 781, 50 m a.s.l.

Diagnosis. Habitus illustrated in Fig. 10A. Distinct from the other species of the genus by the following characters: small size of the body (7.09–7.36 mm); lateral sides of pronotum slightly concave in the anterior part before the maximal width; lateral sides of the mesothoracic pedunculus almost parallel on the anterior third of its length; long hairs on the dorsal surface of the elytra; aedeagus small, median lobe with acute apex, parameres strongly arched inwards, of uniform width, bearing very short setae (Fig. 2G, H).

Description. Body length from 7.09 to 7.36 mm (HT 7.09). General morphology leptodiroid; head wider than pronotum; eyeless; elytra physogastric; wingless, and depigmented.

Head. hypognathous, wider in the anterior part and wider than the pronotum, HL 1.34–1.39 mm (HT 1.39 mm), HW 0.75–0.78 mm (HT 0.78 mm). Surface smooth and shiny with superficial microreticulation and with sparse and short hairs. Few random punctures present on clypeus and along both sides of fronto-genal sulcus.

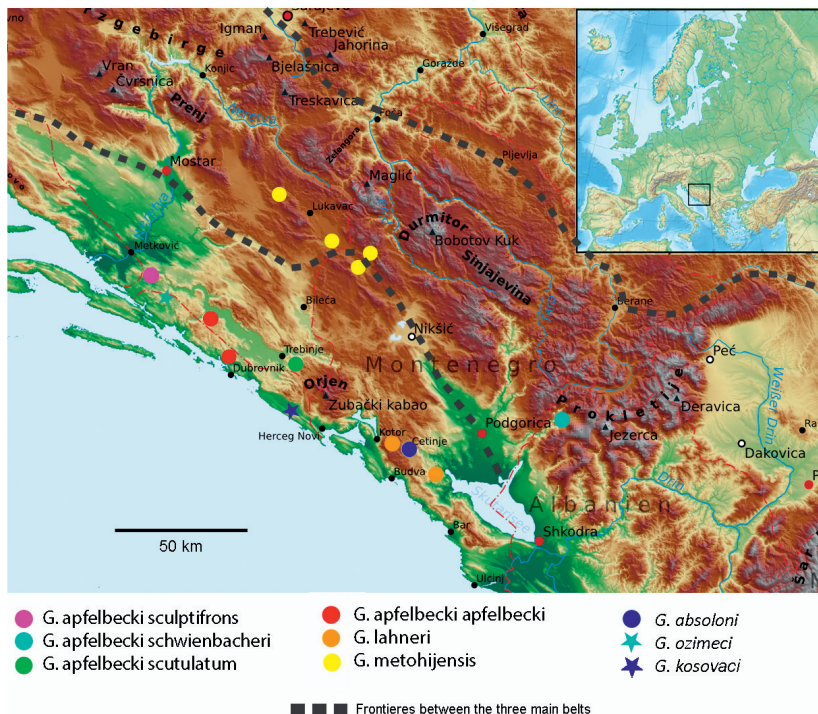


Fig. 9. Distribution map of the species of *Graciliella* in relation to the main geomorphological units of the Dinaric Mountains. Dotted lines separate the three main belts, from south to north: Maritime Belt or Southwestern Belt, the Central Belt or High Dinaric Mountains, and the Northeastern Belt.

Table 3. Antennal formula of the holotype of *Graciliella kosovaci* sp. n.

Article	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Length (mm)	0.47	0.30	1.02	0.86	1.21	1.08	1.00	0.79	0.88	0.75	1.03

Table 4. Antennal formula of the holotype of *Graciliella ozimeci* sp. n.

Article	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Length (mm)	0.51	0.37	1.00	0.92	1.28	1.11	1.07	0.85	0.93	0.76	1.06

Pronotum. Narrow and elongated, approximately three times longer than wide, mean PL/Pwmax 3.06 (HT 3.11). Sides divergent and slightly concave in the anterior part before the maximal width, and convergent between the maximal and minimal width of the pronotum. After the minimal width of pronotum, sides are divergent and with rounded posterior margin. Dorsal surface shiny, with superficial microreticulation, and numerous, random and shallow punctures; hairs are not present.

Mesothoracic pedunculus. 1.16 times (HT 1.09) wider than long; lateral sides parallel in the anterior third of the length (measured from the posterior margin of pronotum to the posterior margin of écusson). Écusson rough, ‘U’-shaped and with microsculptural mesh resembling honeycomb pattern.

Elytra. Physogastric and thick in lateral view, maximal width slightly posterior of the mid-section; mean EL/EW: 1.45 mm (HT 1.52). Surface smooth and shiny, microreticulation not visible; punctuation sparse and superficial. Hairs, sparse and present mostly on the dorsal surface, very rare laterally; as long as the medium-sized hairs on the antennae.

Legs. Like in other species of this genus (see genus diagnosis), protibia straight.

Aedeagus. Small, median lobe longer than parameres (setae included) and with acute apex in dorsal view (Fig. 2G, H). Parameres strongly arched inwards, of uniform width and bearing three short apical setae.

Distribution. Known only from the type locality (Fig. 9). Kaverna 781 is 142 m long and located in south Croatia, in the tunnel ‘Konavosko polje-Sea’ and is probably a part of the upper floor system of underground channels which naturally drain the water of Konavle polje to the sea. Lack of stalactite deposits indicates that the cave is a relatively young foundation, and

the remains of the freshwater cave-dwelling tube worm *Marifugia clavatica* observed on the rock indicates its recent hydrological activity (Cvitanović, 2015).

Etymology. We are glad to dedicate this interesting species to a dear friend, speleologist Petar Kosovac from Belgrade, in recognition for his enthusiastic help in the field and in collecting material.

***Graciliella ozimeci* sp. n.**

urn:lsid:zoobank.org:act:37DB2F08-D301-4637-9023-E948491ABEAA

Type series. Holotype male labeled as follows: ‘CROATIA, Dubrovačko primorje, Točionik, Vranja peć, 230 m, 08.11.2014., leg. R. Ozimec’ (white label, printed)/ ‘HOLOTYPUS *Graciliella ozimeci* sp. n. I. Njunjić, M. Perreau, K. Hendriks, M. Schilthuizen and L. Deharveng det. 2015’ (red label, printed), (CNHM). Paratypes: 27♂ 30♀, 08.11.2014, same cave, leg. R. Ozimec, (CNHM, MNHN, CINJ, CMPR, Naturalis). Paratypes are labeled with white, printed locality labels and with red printed labels ‘PARATYPUS *Graciliella ozimeci* sp. n. I. Njunjić, M. Perreau, K. Hendriks, M. Schilthuizen and L. Deharveng det. 2015’.

Type locality. Croatia, Dubrovačko primorje, Točionik, Vranja peć, 230 m a.s.l.

Diagnosis. Habitus illustrated in Fig. 10B. Distinct from the other species of the genus by the following characters: body size 7.13–7.5 mm; elytra more elongated, EL/EW: 1.47–1.64 mm; shorter mesothoracic pedunculus (1.38 times wider than long); surface of the body matte. Aedeagus with rounded apex in dorsal view and almost straight parameres.

Description. Body length from 7.13 to 7.50 mm (HT 7.45). General morphology leptodiroid; head



Fig. 10. Habitus of *Graciliella*. A. *G. kosovaci* sp. n.; B. *G. ozmeci* sp. n. Scale bar is 1 mm (photos by: Theodoor Hijerman).

wider than pronotum; eyeless; elytra physogastric; wingless, and depigmented.

Head. Hypognathous, wider in the anterior part and wider than pronotum, HL 1.35–1.46 mm (HT 1.46 mm), HW 0.75–0.81 mm (HT 0.81 mm). Surface matte, with superficial microreticulation and with sparse and short hairs on clypeus, genae, and frons, particularly along the dorsal side of fronto-genal sulcus. Last antennomere is shorter in females.

Pronotum. Narrow and elongated, mean PL/Pw-max 2.85 (HT 3.01). Sides almost straight in the anterior part before the maximal width; posterior margin almost straight. Dorsal surface matte and hairless, with superficial microreticulation, and numerous, random and shallow punctures. Ventral side of the pronotum completely smooth behind the first pair of legs.

Mesothoracic pedunculus. 1.38 times (HT 1.53) wider than long; lateral sides parallel in the anterior quarter of the length (measured from the posterior margin of pronotum to the posterior margin of écusson). Ecusson rough, 'U'-shaped and with microsculptural mesh resembling honeycomb pattern.

Elytra. Physogastric but narrow; maximal width slightly posterior of the mid-section; mean EL/EW: 1.47–1.64 mm (HT 1.49). Surface irregular and matte, microreticulation and punctures visible in some specimens. Hairs very sparse and present mostly on the dorsal surface, very rare or absent laterally.

Legs. Like in other species of the genus (see genus description), protibia straight.

Aedeagus. Median lobe longer than parameres (setae excluded) and with rounded apex in dorsal view (Fig. 2I, J). Parameres almost straight, of uniform width and bearing three apical setae.

Distribution. Known only from the type locality (Fig. 9). Vranja peć is situated in south Croatia, in the municipality Dubrovačko primorje, close to the village Točionik. It is a cave with a vertical entrance, 188 m long and 50 m deep. The bottom of the channel is covered with rock debris and large blocks of rocks broken off from the ceiling. The cave is hydrologically inactive except the small drippings at the bottom (Cvitanović, 2015).

***Graciliella apfelbecki apfelbecki* (Müller, 1910) n. comb.**

Anthroherpon apfelbecki Müller, 1910: 186.

Type locality. Höhle zwischen Jasenica und Zavala (Herzegowina) [= pećina Vjetrenica; N 42° 50' 28.34" E 17° 59' 11.87", alt. 268 m]

Anthroherpon Apfelbecki J. Müller: Jeannel, 1911: 560.
Anthroherpon (s. str.) *Apfelbecki* J. Müller, 1910: Jeannel, 1924: 420.

Anthroherpon Apfelbecki Müll: Winkler, 1925: 144.

Anthroherpon (s. str.) *Apfelbecki* subsp. *Apfelbecki* Müller: Jeannel, 1930: 147.

Anthroherpon apfelbecki J. Müller: V.B. Guéorguiev, 1990: 264.

Anthroherpon apfelbecki apfelbecki J. Müller, 1910: Giachino and Guéorguiev, 1993: 316.

Anthroherpon apfelbecki J. Müller: Perreau and Pavićević, 2008: 211.

Anthroherpon Kauti Apfelbeck, 1911: 216. (synonymy in Jeannel, 1914).

Type locality: Herzegowina: in der Vjeternica höhle bei Zavala.

Anthroherpon Kauti Apfelbeck, 1916: 346.

Material examined. 1♂: LECTOTYPE (here designated), Hercegovina, 'Vjeternica H.', 11.07.1914., leg. K. Absolon, RMNH.INS.627472 (Naturalis); 1♀: PARALECTOTYPE (here designated), Herzegovina Vjeternica, 1931, leg. K. Absolon (MNHN); 1♂, 1♀: Herzegovina Vjeternica, leg. K. Absolon (MNHN); 1♂, 1♀: Herzegovina Vjeternica, leg. K. Absolon (MNHN); 1♀: Zavala, Hr., leg. Novak, 5.9.1925 (MNHN); 1♀: Zavala, Herzeg. (MNHN); 1♂: Hercegovina, Neretva, Ravno, Zavala, Vjeternica pećina, Popovo polje, 42.88–17.98 03/VI/2011., leg. M. Perreau, RMNH.INS.550268 (Naturalis); 1♀: same data, RMNH.INS.550269 (Naturalis); 1♀: Vjeternica pećina, Collectie G. Paganetti-Hummeler acq. 1937, ZMA.INS.1236005 (Naturalis); 1♀: same data, ZMA.INS.1236007 (Naturalis); 1♂: Hercegovina, Zavala, Dr. Grabowski, Coll. Dr. S. Breuning, ZMA.INS.1235988 (Naturalis); 2♀: BiH, Zavala, Vjeternica., leg. R. Ozimec (CNHM); 1♀: same locality, 21.08.2008., leg. Bakšić Darko (CNHM); 2♂: same locality, 20.08.2007., leg. M. Perreau (CMPR); 1♀: Zavala, Popovo polje, Vjeternica, Hercegovina, 12.09.1975., leg. Pretner (CMPR); 1♀: Dr. Absolon, Vjeternica (CMPR); 1♂: Vjeternica, Absolon, 1912, Hercegovina (CMPR); 1♀: Kali pećina apud Grepci, Hercegovina, Absolon (CMPR); 3♀: Grabovica bei Grepci, Hercegovina, Absolon (CMPR); 3♂ 3♀: Gjurkovina pećina apud Grepci, Hercegovina, Absolon (CMPR); 2♂ 4♀: Močiljska pec, Rjeka-Dubrov., Svircev, 5.1.931. (MNHN); 1♂ 1♀: Močiljska pec. Dubrovnik, Svircev, 1.9.923. (MNHN); 2♂: Croatia, Dubrovačko primorje, Dubrovnik, Osojnik, Močiljska špilja, 12.11.2014., leg. Hanžek Nikola (CNHM); 2♀: same locality, leg., R. Ozimec (CNHM); 1♀: Močiljska pećina, Močilje, Ombla, Dalmatia, leg. E.

Pretner (CMPR); 1♂: Croatia, Dubrovačko primorje, Dubrovnik, Gromača, Špilja za Gromačkom vlakom, 28.08.2014., leg. Slapnik Rajko (CNHM); 1♀: same locality, 25.09.2014., leg. Slapnik Rajko (CNHM); 1♀: Bosnia and Herzegovina, Bobani, Grabovica, 08.05.2011., leg. R. Ozimec (CNHM); 1♀: Grotte Grabovica, Herzegovina Weirather, 1-14 (MNHN); 1♂: Kali pećina, Grebci, Herceg., Svircev, 2.1.1931. (MNHN); 1♂: Gr. Močilje, pr. Ragusa, 30.11.1921., G. Müller (MNHN); 2♀: Orlica pećina, Popovo polje, Herzegovina, Absolon (CMPR); 1♀: Ravno Hercegov. VI. Zoufal (CMPR); 1♂: Bihovo, Trebinje, Herz, Collectie G. Paganetti-Hummler acq. 1937, ZMA.INS.1236013 (Naturalis).

Diagnosis. Habitus illustrated in Fig. 11A. Body length from 7.3 to 8.63 mm. Median lobe with lanceolate apex in dorsal view and considerably longer than parameres (setae excluded). Parameres of uniform width, slightly curved inwards in the apical part and bearing three long apical setae (Fig. 2A, B).

Remarks. Two specimens, one in Naturalis and the other one in MNHN were labelled as types, so we designated the male specimen from Naturalis as lectotype and female specimen from MNHN as paralectotype.

We do not have molecular data for the three following subspecies of *G. apfelbecki*, so we follow the classification of Giachino and Vailati (2005) on this point.

***Graciliella apfelbecki scutulum* (Giachino and Guéorguiev, 1993) n. comb.**

Antroherpon scutulum Giachino and V.B. Guéorguiev, 1993: 317.

Type locality: Erzegovina, Bravenik [= Hercegovina, Zupci, Bravenik; N 42°37.143' E 18°25.969', alt. 740 m a.s.l.]

Antroherpon apfelbecki scutulum Giachino and Guéorguiev: Perreau and Pavićević, 2008: 212.

Material examined: 1♂ 3♀: BiH, Trebinje, Zupci, Bravenik (= Jama u Braveniku), alt. 740 m a.s.l., 19.06.2003., leg. M. Perreau (CMPR).

***Graciliella apfelbecki schwienbacheri* (Giachino and Vailati, 2005) n. comb.**

Antroherpon apfelbecki schwienbacheri Giachino and Vailati, 2005: 159.

Type locality: Albania, Kelmend, Tamara umg., Shpella Siper Rruges, 185m a.s.l.

Material examined: We did not examine this subspecies.

***Graciliella apfelbecki sculptifrons* (Winkler, 1925) n. comb.**

Antroherpon Apfelbecki sculptifrons Winkler, 1925: 144.

Type locality: In einem Hallenschacht bei der Žaba planina südlich von Metković [=Žaba Mt, Golubinka]

Antroherpon (s. str.) *Apfelbecki* subsp. *sculptifrons* Winkler: Jeannel, 1930: 147.

Antroherpon apfelbecki sculptifrons Winkler: Pretner, 1974: 11.

Material examined: 1♂: TYPE, Žaba plan., Golubinka (NHM).

***Graciliella metohijensis* (Zariquiey, 1927) n. stat., n. comb.**

Antroherpon Apfelbecki subsp. *Metohijensis* Zariquiey, 1927: 163.

Type locality: Höhle 216, Urvol P., Nordostherzeg., Karstlande [= Đatlo pećina, Korita, Bosnia and Herzegovina; N 43°03.710' E 18°29.678', alt. 1013 m a.s.l.]

Antroherpon (s. str.) *Apfelbecki* subsp. *metohijensis* Zariquiey: Jeannel, 1930: 147.

Antroherpon apfelbecki metohijense Zariquiey: Pretner, 1974: 11.

Antroherpon apfelbecki metohijensis Zariquiey: Guéorguiev, 1990: 265.

Antroherpon apfelbecki metohijensis Zariquiey, 1928: Giachino and Guéorguiev, 1993, Natura bresciana: 317.

Material examined. 1♂: TYPE, H 216 Urvol p. Weirather, NordostHerzeg. 1922., Karstlande (MNHN); 1♂: BiH, Korita, Kobilja Glava, Đatlo, alt. 1013m, 13.09.2006., leg. M. Perreau (CMPR); 1♀: same locality, 05.08.2013., leg. M. Perreau (CMPR); 1♀: same locality, 15.03.2008, leg. S. Ognjenović (CMPR); 1♀: same locality, 13.07.2007., leg. M. Đokić (CMPR); 3♂: same locality, 23.06.2003., leg. M. Perreau (CMPR); 3♂ 3♀: BiH, Korita, Brestica, Jametina, 14.07.2007., leg. M. Perreau (CMPR); 10♂ 8♀: BiH, Balabani, Drvendžina pećina, 26.08.2013., leg. I. Njunjić (CMPR, CINJ); 3♂ 3♀: Montenegro, Golija Mt, Donje Čarađe, Prljača, 07.08.2010., leg. S. Ognjenović (CDPV); 2♂, same locality, 28.08.2010., leg. I. Njunjić (CINJ); 2♀, same locality, 13.08.2010, M. Plečaš (CDPV); 1♂, same locality, same date, leg. M. Perreau (CMPR); 3♀: Montenegro, Kazanci, Bijela pećina, 10.08.2010, leg., M. Perreau; 1♀: Hercegovina, grot Provalija, 22.07.1962., RMNH.INS.627543 (Naturalis); 1♀: same

data, Hercegovina, grot Provalija, 22.07.1962., RMNH. INS.627700 (Naturalis); 1♂ 5♀: Montenegro, Stozi, Stoška pećina, 10.08.2010., leg. I. Njunjić (CINJ); 2♀: same data, leg. S. Ognjenović (CDPV).

Diagnosis. Habitus illustrated in Fig. 11B. Large species, body length from 7.76 to 8.53 mm. Distinct from the other species of the genus by the following characters: elytra strongly physogastric and very wide, mean EL/EW: 1.33, with the largest width slightly before the mid-section and dense but shallow punctuation. Sparse hairs are present mostly on the dorsal surface of the elytra. Pronotum with rounded anterior margin. Parameres shorter than the median lobe (setae excluded), with strong constriction in the preapical region (Fig. 2C, D) in some populations, or without the constriction. Median lobe of the aedeagus with lanceolate apex in dorsal view. This combination of characters supports the change of the status from subspecies to species rank. New status is consistent with the molecular phylogeny, morphometrics and geographic location of this species mostly in the Central Belt of the Dinaric Mountains (Golija Mt., Vojnik Mt., Crvanj Mt., Somina Mt., Korita), rather than in the Southwestern (Maritime) Belt as most of the other species and subspecies of the genus *Graciliella*.

Remarks. There is inter- and intrapopulation variability in the shape of parameres: In some populations (e.g., Drvendžina pećina in Balabani) the parameres have a strong preapical constriction while in others (e.g., Prljača on Golija Mt., Stoška pećina on Somina Mt.), the parameres are of equal width in full-length. In the population from Drvendžina pećina there is a gradient in this character: from evident preapical constriction to parameres without constriction. Some specimens from this population have asymmetric aedeagi: one paramere with constriction and other without. Since we have sequences from only two specimens from the population of Drvendžina pećina, we are unable to assess the taxonomic significance of this variability.

***Graciliella lahneri* (Matcha, 1916) n. stat. n. comb**
Anthroherpon Lahneri Matcha, 1916: 63.

Type locality: Montenegro: Lottspeich Grotte bei Njeguši

Anthroherpon (s. str.) *Lahneri* Matcha, 1916: 420.

Anthroherpon Lahneri Matcha: Fagniez, 1927: 23.

Anthroherpon (s. str.) *Lahneri* Matcha: Jeannel, 1930: 147.

Anthroherpon apfelbecki lahneri Matcha: Guéorguiev, 1990: 265.

Anthroherpon apfelbecki lahneri Matcha, 1916: Giachino and Guéorguiev, 1993: 317.

Material examined. 1♀: TYPE, Lovćen, Geb. Mal. 1916, Montenegro, Rittm. Matcha, Duboki do (MNHN); 1♀: Montenegro, Lovćen Mt, Bjeloši, Jankova jama, 27.06.2013., leg. I. Njunjić (CINJ); 2♂: Montenegro, Virpazar, Trnovo, Grbočica, 30.05.2004., leg. R. Mlejnek (CMPR); 1♂: Montenegro, Virpazar, Trnovo, Grbočica pećina, cave traps, 21.03-16.07.2008., leg. R. Lohaj, ZMA.INS.1236016 (Naturalis); 1♀: same data, ZMA.INS.1235992 (Naturalis).

Diagnosis. Elytra slightly convex on the disc. Median lobe of the aedeagus elongated and narrow, with acute apex. Parameres as long as the median lobe, with weak preapical constriction and significantly wider in the apical part. Apex of the parameres acute and bearing three setae (Fig. 2L, M). The distinctive shape of the aedeagus allows the change of the status from subspecies to species rank. New status is consistent with the morphometrics and the geographic location of this species in Mountains of Maritime Montenegro.

***Graciliella absoloni* (Guéorguiev, 1990) n. comb.**
Anthroherpon absoloni Guéorguiev, 1990: 265.

Type locality: Montenegro, Lipska pećina près de Cetinje [N 42° 22.433' E 018° 57.207', alt. 456 m a.s.l.]

Material examined. 1♂: TYPE, Montenegro, Lipska pećina près de Cetinje, 10.VIII 1912., leg. J. Matcha (NMP); 1♀: PARATYPE, same data (NMP).

Diagnosis. Body length 7.53 mm (HT). Elongated mesothoracic pedunculus, narrowest in the anterior part, PL/MP: 4.13 (HT). Pronotum distinctly longer than wide, narrow, and with a strong constriction in the posterior quarter. Maximal width is in the second third of its length; PL: 1.98 mm (HT). Elytra elliptic and physogastric; EL: 3.5 mm (HT). Aedeagus slender, endophallus without sclerotized structures, elongated and with lanceolate apex in dorsal view. Parameres shorter than the median lobe, with weak preapical constriction and slightly wider in the apical part, bearing three setae (Fig. 2E, F). These characters fit in the description of *Graciliella* which allows us to place this species in this new genus.



Fig. 11. Habitus of *Graciliella*. A. *G. apfelbecki apfelbecki*; B. *G. metohijensis*. Scale bar is 1 mm (photos by: Theodoor Hijerman).

Identification key

Here we present the identification key to the genus *Graciliella*, excluding the subspecies. It is dedicated to practical identification of species and does not correspond to the molecular phylogeny. Although we have used a few characters previously mentioned in the *Anthroherpon* key of Giachino and Vailati (2005), the present key is based chiefly on the diagnoses provided above.

1. Elytra strongly physogastric and very wide, EL/EW: 1.18-1.43 mm, with the largest width slightly before the middle, depressed and attenuated towards the apex *metohijensis* (Zariquiey)
- Elytra less physogastric and less wide, EL/EW: 1.3-1.64 mm, with the largest width in the mid-section or slightly in the posterior part of the mid-section, not depressed and not attenuated towards the apex 2
2. Anterior part of the pronotum ovoid. Parameres as long as the median lobe, significantly wider in the apical part *lahneri* (Matcha)
- Anterior part of the pronotum subcylindrical. Parameres shorter than the median lobe, same width in the full length or slightly wider in the apical part ... 3
3. Protibiae weakly curved inwards. Elytra with rounded apex and with the largest width in the middle. Parameres with weak preapical constriction and slightly wider in the apical part. (6.7-7.1 mm) .. *absoloni* (Guéorguiev)
- Protibiae straight. Elytra with acute apex and with the largest width slightly posterior of the mid-section. Parameres of uniform width, without constriction in the apical part 4
4. Lateral sides of pronotum convex in the anterior part. Median lobe with lanceolate apex in dorsal view and considerably longer than parameres (setae excluded). Parameres of uniform width, slightly curved inwards in the apical part. Body length 7.3-8.63 mm *apfelbecki* (Müller)
- Lateral sides of pronotum concave or almost straight in the anterior part. Median lobe with acute apex or rounded in the apical part, longer than parameres (setae excluded). Parameres strongly arched inwards or almost straight. Body length 7.09-7.5 mm 5
5. Lateral sides of the pronotum concave in the anterior part before the maximal width. Dorsal surface of the body smooth and shiny. Long hairs on the

dorsal surface of the elytra. Aedeagus with acute apex in dorsal view, parameres strongly arched inwards, bearing very short setae *kosovaci* sp.n.

- Lateral sides of pronotum almost straight in the anterior part before the maximal width. Dorsal surface of the body matte. Short hairs on the dorsal surface of the elytra. Aedeagus with rounded apex in dorsal view, parameres almost straight, bearing long setae *ozimeci* sp.n.

Biogeography and ecology

The genus *Graciliella* is distributed in subterranean habitats of the Dinaric Mountains, from Crvanj mountain (Bosnia and Herzegovina) in the north, to Trnovo (Montenegro) in the south, and from Žaba mountain (Croatia) in the west to Prokletije mountain (Montenegro) in the east. It is distributed from an altitude of 50 m (*G. kosovaci* in Croatia, Konavle, Kaverna 781) to 1270 m (*G. metohijensis* in Montenegro, Somina, Stoška pećina) above sea level.

Figure 9 shows the geographic distribution of the species of the genus *Graciliella*, in relation to the three main geomorphological units of the Dinaric Mountains: the Maritime Belt or Southwestern Belt, the Central Belt or High Dinaric Mountains, and the Northeastern Belt (Hajna, 2012). The genus *Graciliella* is present only in the Maritime and Central Belt. Even though the cave fauna of the Northeastern Belt is fairly well known, *Graciliella* have never been found in this region. The distribution of *G. apfelbecki schwiebacheri* far from other *apfelbecki* subspecies and in the Central Belt rather than in the Maritime Belt of the Dinaric Mountains where other *apfelbecki* subspecies are distributed indicates that the systematic position of this subspecies should be analyzed using molecular approaches.

In the region of Velež Mt. in Bosnia and Herzegovina all three genera were found – *Leptomeson loreki* Zoufal, 1904, *Anthroherpon ganglbaueri ganglbaueri* (Apfelbeck, 1894) and *Graciliella metohijensis* (Zariquiey, 1927), but never in syntopy. However, in a few caves in Bosnia and Herzegovina (e.g. Vjeternica in Zavala, Bravenik in Zupci, Veliko Đatlo in Korita) and Croatia (Špilja za Gromačkom Vlakom in Dubrovačko primorje), *Graciliella* and *Hadesia* coexist in the same cave. The biotope of *Hadesia* differs in many aspects from most other troglobitic species of Leptodirini – these beetles inhabit a semi-aquatic cave habitat called ‘hygropetric’ – they are always found in the vicinity of strong flows of running water

on stalagmitic walls and often inside the film of running water (Jeannel, 1924, Remy, 1940, Sket, 2004, Perreau and Pavićević, 2008). *Graciliella*, *Anthroherpon* and *Leptomeson*, however, prefer cave walls without water flow and they have never been found inside the water film itself. Also, they have never been observed on deposits of guano and almost never under the rocks on the cave floor. The representatives of these three genera inhabit humid cave walls, stalac-

tites and stalagmites with almost no visible siltation. Their diet is understudied but it is assumed that they are saprophagous like many other Cholevinae (Beutel and Leschen, 2016) while *Hadesia* is considered to filter water or brush the surface of stalagmitic walls with its specialized mouthparts (Perreau and Pavićević, 2008). The different ecological niches in *Hadesia* and *Graciliella* may allow coexistence without competition.

